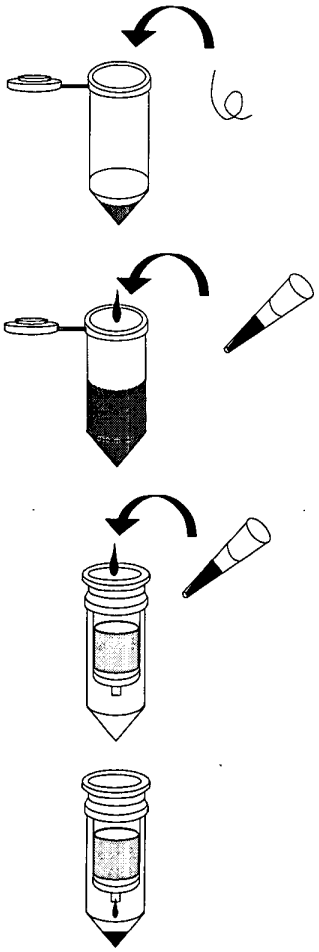
Figure 1.

Extraction of Mitochondrial DNA by the Method of the Invention



1. Add washed hair shaft to 50 ul of Lysis Buffer
2. Incubate 10 Minutes @ 65°C
3. Add Binding Buffer and mix
4. Load solution onto Binding Column and spin 1 minute
5. Add Wash Buffer to column and spin 1 minute
6. Add Elution Buffer to column and spin 1 minute
7. Recover pure mitochondrial DNA in 75 ul water

Extraction of Mitochondrial DNA by the method of Wilson et al.

1. Place washed hair fragment into tissue grinder.
2. Grind the hair until fragments are no longer visible.
3. Transfer the homogenate to a sterile 1.5 ml plastic tube.
4. Add 1 ul of 600U/ml Proteinase K to each tube.
5. Vortex on low speed and briefly centrifuge.
6. Place tubes in water bath and incubate at 56°C for 24 hours.
7. Add 200 ul of phenol/chloroform/isoamyl alcohol (PCIA) to each tube.
8. Vortex 30 seconds to obtain a milky emulsion.
9. Spin tubes at 10,000 x g for 3 minutes to separate phases.
10. Assemble and label a Microcon™ 100 unit.
11. Add 200 ul deionized water to the filter side (top) of each concentrator.
12. Carefully remove the aqueous phase (supernatant) and transfer to the concentrator. Avoid drawing any of the proteinaceous interface into the pipette tip.
13. Spin the Microcon™ 100 concentrators for 5 minutes at 3000 x g.
14. Discard the filtrate and return the filtrate cups to the concentrators.
15. Add 400 ul of deionized water to the filter side of each concentrator.
16. Spin again at 3000 x g for 5 minutes and discard the filtrate cups.
17. Add 60 ul of hot (80-90°C) deionized water to the filter side of each concentrator and place a retentate cup on the top of each concentrator.
18. Briefly vortex the concentrators with the filtrate cups pointing up.
19. Invert each concentrator with its retentate cup and spin in a microcentrifuge at 10,000 x g for 3 minutes.
20. Discard the concentrators. Cap the retentate cups containing the mitochondrial DNA.

Figure 2. Extraction of mitochondrial DNA from Dog, Human, Cat and Horse Hair containing no root.

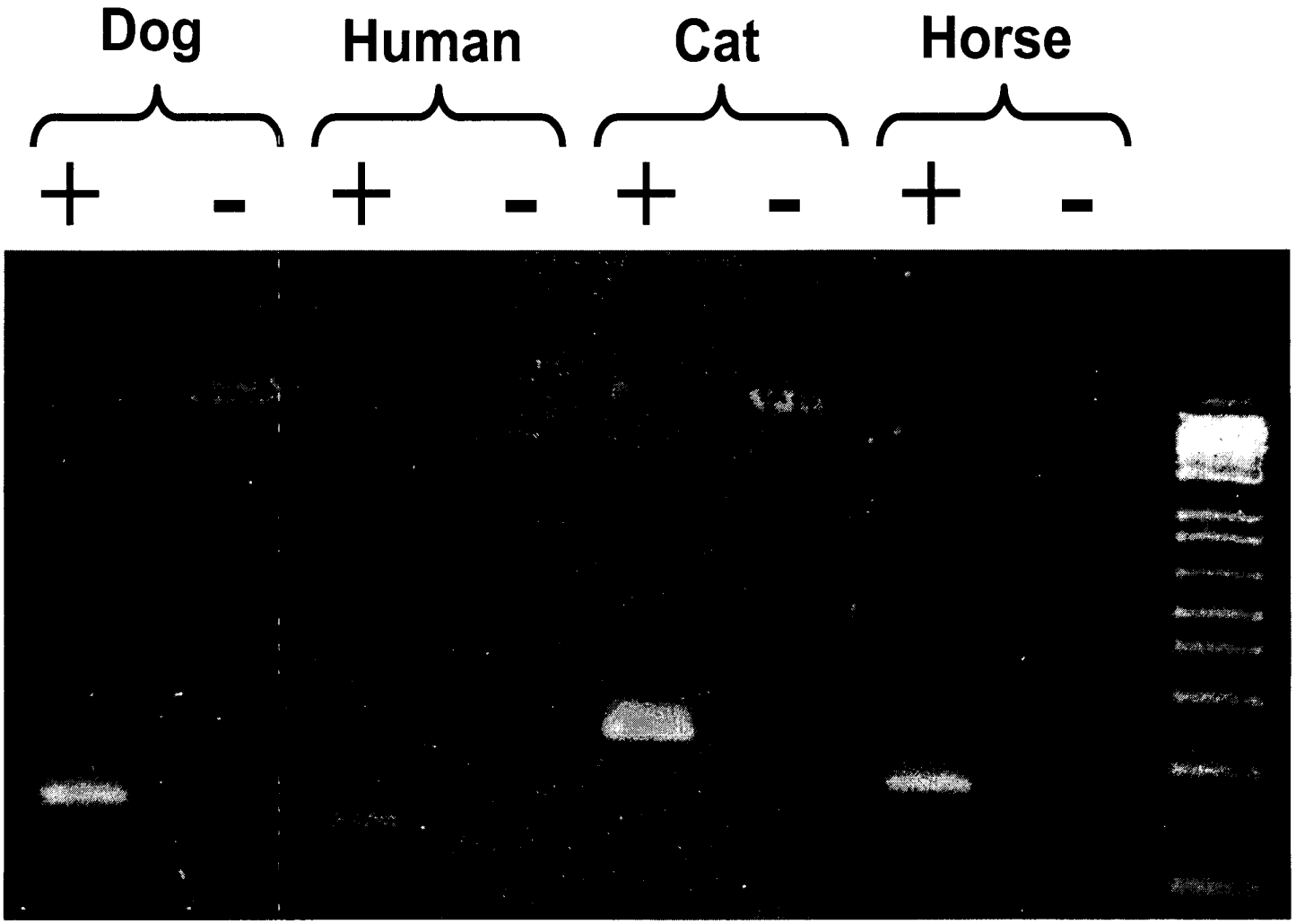


Figure 3.

Relative signal intensity of each allele obtained after PCR amplification and detection of mitochondrial DNA sequences from five samples obtained from the same person.

			Relative Intensity					
			Locus 73		Locus 93		Locus 263	
Sample	**Description**	**Method of DNA Extraction**	**A**	**G**	**A**	**G**	**A**	**G**
Sample 1	2.0 cm hair shaft from head with no root attached	The method of the invention	16%	**84%**	**82%**	18%	22%	**78%**
Sample 2	2.0 cm pubic hair with no root attached	The method of the invention	18%	**82%**	**84%**	16%	14%	**86%**
Sample 3	2.0 cm pubic hair with no root attached	Proteinase K, phenol chloroform (Wilson et al.)	20%	**80%**	**83%**	17%	12%	**88%**
Sample 4	2mm punch of blood-spotted FTA paper	The method of the invention	20%	**80%**	**85%**	15%	11%	**89%**
Sample 5	Whole blood	Qiamp whole blood mini kit (Qiagen Corp)	14%	**86%**	**78%**	22%	27%	**73%**